Induction of Vascular Endothelial Growth Factor Expression in Endothelial Cells by Platelet-derived Growth Factor through the Activation of Phosphatidylinositol 3-Kinase

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ABSTRACT

Increased numbers of platelet-derived growth factor β receptors (βPDGFRs) on neovascular endothelial cells is a common occurrence in several pathological conditions including wound healing, inflammation, and glioma tumorigenesis. Here we sought to test the biological significance of this by determining whether expression of wild-type βPDGFR by normal aortic endothelial cells affected the expression of the vascular endothelial growth factor (VEGF), a critical angiogenesis regulator and mitogen for such cells. The results showed that PDGF could increase transcription and secretion of VEGF by βPDGFR-expressing endothelial cells. Moreover, we further demonstrated a requirement for the activation of phosphatidylinositol 3-kinase (PI3K) in this response by using chemical inhibitors of PI3K, mutant PDGFR, and dominant-negative PI3K. These studies suggest a novel mechanism by which PDGF induces VEGF expression in endothelial cells, define VEGF as a downstream target for PI3K, and invoke a role for PI3K in angiogenesis.

INTRODUCTION

The VEGF is a potent endothelial cell-specific mitogen that plays a critical role in angiogenesis (reviewed in Refs. 1–3). VEGF is a homodimeric glycoprotein of 23 kDa subunits that is evolutionarily conserved and shares homology with placental growth factor and PDGF. Its biological effects are elicited through two high affinity receptor tyrosine kinases (Flt-1/VEGFR-1 and KDR/VEGFR-2). In addition to mitogenesis, VEGF also induces vascular permeability, intracellular Ca2+ influx, chemotaxis, and increased expression of plasminogen activators, the urokinase receptor and collagenases (2). The temporal and spatial correlation of VEGF and its receptors with angiogenesis during embryonic development, tumor growth, inflammation, and wound healing indicates its role as a key physiological and pathological mediator of angiogenesis (4–10). This assertion is directly supported by two lines of evidence: (a) targeted disruption of the genes for either the VEGF ligand or its receptors results in severe defects in the developing vasculature, ultimately resulting in embryonic lethality (11–13); and (b) interference with the VEGF/ receptor system by specific antibodies, anti-sense VEGF, or a dominant-negative VEGF receptor flk-1/VEGFR-2 mutant results in significant inhibition of neovascularization and tumor growth in experimental systems (14–16). Several biologically relevant agents and conditions have been shown to induce VEGF expression in various types of cells.

Received 10/16/98; accepted 1/29/99.

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levels of receptor expression were obtained by fluorescence-activated cell sorting, using a human-specific anti-βPDGFR monoclonal antibody (Genzyme) with FITC-conjugated anti-mouse IgG (PharMingen) as the secondary antibody. The sorted cell populations were grown in G418-containing media and used for all experiments described here.

Generation of Stable Cell Lines Expressing Dominant-Negative PI3K.

The p85-based-dominant negative mutant of PI3K (p85 ∆SH2ΔN, deletion of amino acids 494–790) in pSG5 (38, 39) was kindly provided by Drs. M. Waterfield and J. Downward (Ludwig Institute for Cancer Research, London, and Imperial Cancer Research Fund, London, respectively). The EcoRI fragment of the p85 ∆SH2ΔN excised from pSG5 was subcloned into pcDNA3.1 Zeroxin (Invitrogen). The plasmids were transfected into PAE/βPDGFR cells using Lipofectamin reagent (Life Technologies, Inc.). Individual zeroxin-resistant clones were selected and analyzed by Western blotting for the expression of both the mutant p85 and βPDGFR.

Northern Blot Analysis.

Cells were grown to 90% confluency and starved in SFM containing 0.1% BSA (SFM/BSA) for 18–20 h. After stimulation with PDGF-BB (R&D System) at indicated concentrations, cells were harvested, and total cellular RNA was isolated using the Trizol reagent (Life Technologies, Inc.). For drug treatment experiments, cells were preincubated either with the vehicle, DMSO (final concentration, 0.01%) alone, or the following drugs dissolved in DMSO for 30 min before the addition of PDGF or TPA (Sigma): 5 μg/ml actinomycin D (Sigma), 5 μg/ml cycloheximide (Sigma), 5 μM bisindolylmaleimide GF 109203X (BIM; Calbiochem), and wortmannin or LY24009 (Calbiochem) at the indicated concentrations. A 15-μg total cellular RNA sample was then fractionated through 1% agarose containing 2.2 M formaldehyde and transferred to Hybond blotting membrane (Amersham). A VEGF165 cDNA probe (0.6 kb) was 32P-labeled by the random primer method and then hybridized to filters in QuickHyb solution (Stratagene). The blots were then exposed to a phosphor imager screen or autoradiography film. The bands were quantified by densitometry.

RESULTS

PDGF Increases Steady-State VEGF mRNA Levels in a Concentration- and Time-Dependent Manner in PAE/βPDGFR Cells. Although expression of βPDGFR is induced on tumor endothelial cells of low- and high-grade gliomas (30, 31), primary cultures of tumor-derived endothelial cells do not maintain receptor expression beyond 2 days in culture (30). To overcome this and to determine whether βPDGFR mediates VEGF expression in endothelial cells, wild-type βPDGFR was introduced into normal PAE in which endogenous βPDGFR expression was not detectable (Fig. 1A). Although VEGF mRNA was not detectable in parental PAE cells (not shown) or PAE cells expressing the empty vector (Fig. 1B), exposure to VEGF resulted in substantial induction of VEGF mRNA (Fig. 1A, 1B, first lane) without stimulation. This was probably due to an autocrine induction of VEGF by a small amount of PDGF expressed by the PAE cells because PDGF-BB neutralizing antibody (R&D Systems) blocked such induction (data not shown). Exposure of PAE/βPDGFR cells to exogenous PDGF-BB caused a further and substantial induction of VEGF mRNA (Fig. 1, B and C, first lane) and protein (Fig. 5B, first lane) without stimulation. This was probably due to an autocrine induction of VEGF by a small amount of PDGF expressed by the PAE cells because PDGF-BB neutralizing antibody (R&D Systems) blocked such induction (data not shown). Exposure of PAE/βPDGFR cells to exogenous PDGF-BB caused a further and substantial induction of VEGF mRNA (Fig. 1, B and C). Similar results were observed in human umbilical vascular endothelial cells that express exogenous βPDGFR (data not shown). Basal levels of VEGF mRNA were increased by 1 ng/ml PDGF-BB and reached a peak level by 20 ng/ml in a concentration-dependent fashion (Fig. 1C). Kinetic studies using 50 ng/ml PDGF-BB revealed that maximal induction was achieved by 2 h and then started to decrease at 4 h, gradually returning to basal level by 12 h (Fig. 1B). Taken together, these results showed that introduction of βPDGFR into endothelial cells created an autocrine loop with endogenous PDGF, resulting in VEGF induction by the cells. Exposure of these cells to exogenous PDGF further augmented VEGF induction in a concentration- and time-dependent manner.

Up-Regulation of VEGF mRNA Correlates with VEGF Protein Accumulation in the CM. To determine whether the observed up-regulation of VEGF mRNA levels by PDGF led to a coordinate induction of VEGF proteins, CM from PDGF-treated and untreated PAE/βPDGFR cells was analyzed for the presence of VEGF protein. For immunoblot analysis, CM was enriched and partially purified by
PDGF-induced VEGF Stimulates Endothelial Cell Mitogenesis. To test whether the VEGF protein in the CM was capable of stimulating endothelial cell mitogenesis, we examined BrdUrd incorporation into cellular DNA in PAE/KDR cells after CM treatment. As PAE/KDR cells expressed little, if any, VEGF protein, untreated PAE/βPDGFR cells expressed an elevated VEGF protein, also consistent with the low VEGF mRNA levels in these cells (Fig. 1). PDGF-induced VEGF Stimulates Endothelial Cell Mitogenesis. To test whether the VEGF protein in the CM was capable of stimulating endothelial cell mitogenesis, we examined BrdUrd incorporation into cellular DNA in PAE/KDR cells after CM treatment. As PAE/KDR cells expressed little, if any, VEGF protein, untreated PAE/βPDGFR cells expressed an elevated VEGF protein, also consistent with the low VEGF mRNA levels in these cells (Fig. 1).

Binding to heparin-Sepharose. As shown in Fig. 2A, three protein bands were recognized by anti-VEGF antibody: (a) a major band with a molecular mass of 23 kDa was similar to that VEGF165 (Fig. 2A, middle band); (b) an upper band that was most likely VEGF189; and (c) a lower band that appears to be a proteolytic product rather than VEGF121, that lacks heparin-binding domain and was removed from the partially purified samples. For better quantitation, the original nonpurified CM was used for VEGF ELISA. As shown in Fig. 2B, PDGF stimulation of PAE/βPDGFR cells caused a 5-fold increase in VEGF protein accumulation, which correlated with the VEGF mRNA levels in these cells as shown in Fig. 1. Although vector-transfected cells expressed little, if any, VEGF protein, untreated PAE/βPDGFR cells expressed an elevated VEGF protein, also consistent with the low VEGF mRNA levels in these cells (Fig. 1).

PDGF-induced VEGF Expression Is Blocked by PI3K Inhibitors. The signaling cascades from PDGF receptor activation to increased VEGF gene transcription have not been defined in endothelial cells. We focused on the potential of PI3K as a mediator of VEGF induction because of its central involvement in multiple signaling pathways (44). As shown in Fig. 5A, two specific PI3K inhibitors with different modes of action, wortmannin and LY24009, blocked PDGF-induced VEGF expression in a concentration-dependent manner, suggesting that this induction requires PI3K. Because PKC has been shown previously to be essential for PDGF-dependent VEGF induction in NIH3T3 mouse fibroblasts (19), we tested the effects of the specific PKC inhibitor, bisindolylmaleimide GF 109203X (BIM), on VEGF induction in PAE/βPDGFR cells. BIM inhibited TPA-induced VEGF expression (Fig. 5C) but had no inhibitory effect on PDGF-induced VEGF expression; rather, potentiation of the PDGF-induced VEGF up-regulation was apparent (Fig. 5B). Although the mechanism by which this inhibitor potentiated VEGF induction by PDGF is not known, these data demonstrated that the induction was independent of de novo protein synthesis.
INDUCTION OF VEGF IN ENDOTHELIAL CELLS

PKC in endothelial cells. The selective effects of both the PKC and PI3K inhibitors on PDGF- or TPA-induced VEGF expression established their specificity. These results strongly suggested that PI3K, but not PKC, was necessary for PDGF-induced VEGF transcription in PAE/PDGRF cells, whereas the opposite was the case for TPA-induced VEGF expression (Fig. 5C).

Maximal VEGF Induction Requires the Ability of βPDGFR to Activate PI3K. To further test the involvement of PI3K in PDGF-induced VEGF expression, we used mutant βPDGFR forms that had been shown previously to be useful in a dissection of the relative role of each of the β receptor-associated proteins in PDGRF-mediated mitogenic signaling (37). These receptors are shown schematically in Fig. 6A. The F5 βPDGFR mutant lacks five tyrosine phosphorylation sites and consequently does not associate with PLCγ, GAP, SHP-2, or PI3K. The Y40/51 receptor was made by restoring the two tyrosine residues required for docking PI3K back to the F5 receptor, resulting in binding and activation of PI3K but not the other three proteins. The F40/51 receptor lacks the docking sites for PI3K alone and therefore binds all effectors but PI3K (37). R634 is a kinase-defective receptor. Each receptor species was introduced into PAE cells; populations containing similar amounts of cell surface receptors (Fig. 6B, top panel) were isolated by fluorescence-activated cell sorting, and their levels of expression were comparable with that reported in NIH3T3 cells as determined by Western blotting (data not shown).

We first tested the ability of the mutant receptors to associate and activate PI3K upon PDGF stimulation. PDGRF was immunoprecipitated from untreated and PDGF-stimulated cells expressing the wild-type and mutant receptors and analyzed by Western blot analysis and in vitro PI3K assays to measure receptor content, receptor activation by PDGF, and the level of PI3K activity associated with each stimulated receptor. The results showed that the receptors were expressed in each case in roughly equal amounts (Fig. 6, top panel), and that PDGF stimulation resulted in tyrosine phosphorylation of each mutant receptor except the R634 kinase-defective mutant (Fig. 6B, middle panel). The coprecipitation of p85, a regulatory subunit of PI3K, was consistent with this activation, as was the small amount of p85 protein coprecipitated with the F5, F40/51, or R634 receptors (Fig. 6B, bottom panel). As expected, PI3K activity was detected in the immunoprecipitates from activated wild-type and Y40/51 receptors but not from the other mutant receptors (Fig. 6C).

Next we investigated the ability of the mutant receptors to mediate PDGF-driven VEGF transcription. Cells expressing the wild-type and mutant receptors were serum starved and treated with PDGF-BB for 2 h. Steady-state VEGF mRNA levels were analyzed by Northern blotting (Fig. 7A). PDGF-dependent VEGF expression was absent in cells expressing the R634 kinase-defective receptors, indicating that the intrinsic kinase activity of the receptor was a requirement for PDGF-driven VEGF induction. Mutating the tyrosine phosphorylation sites for PI3K binding (F40/51) caused a decrease in VEGF transcription by 3-fold in comparison to the wild-type receptors. Cells expressing the F5 mutant receptors, which lack five tyrosine phosphorylation sites and fail to associate with PI3K as well as other effectors (PLCγ, SHP-2, and GAP; Ref. 37), expressed slightly less VEGF mRNA than the cells expressing the F40/51 receptors, suggesting that receptor-mediated signaling pathway(s) other than PI3K, PLCγ, SHP-2, or GAP may also contribute somewhat to PDGF-dependent VEGF induction. Consistent with this, restoration of the PI3K docking sites to the F5 receptor (Y40/51) resulted in a 2-fold increase in VEGF expression compared with the F5 receptors. It should be pointed out that, as shown in Figs. 1 and 2, the basal level of VEGF mRNA in receptor transfectants increased largely due to an autocrine PDGF induction of VEGF expression. Therefore, the magnitude of VEGF increase upon exogenous PDGF stimulation appears to represent an increased response to doses of PDGF. In this light, the effects of mutant receptors on VEGF induction are best assessed by comparing levels of VEGF mRNA among PDGF-stimulated cells expressing those receptors rather than comparing the magnitude of VEGF induction upon exogenous PDGF stimulation among mutant receptors.

We then determined whether VEGF mRNA induction by PDGF led to a coordinate induction of VEGF proteins in cells expressing these mutant receptors. VEGF proteins secreted into the CM of cells treated with PDGF for 24 h were measured by Western blotting (Fig. 7B, upper panel) and VEGF ELISA (Fig. 7B, lower panel). Only a small amount of VEGF proteins was detected by both methods in the CM collected from cells expressing the F5 or F40/51 receptors. In contrast, a significant increase in VEGF protein accumulation was observed in the CM from the cells expressing Y40/51 receptors, in agreement with the VEGF mRNA data shown in Fig. 7A. Apparently, the effects of the F5 and F40/51 receptors on VEGF protein accumulation in the CM was more profound than on VEGF transcription, possibly due to decreases of VEGF protein translation, secretion, and/or protein stability.

Finally, we determined whether the levels of VEGF proteins in the CM collected from the cells expressing mutant receptors correlated with their activity to stimulate VEGF receptor (KDR)-mediated endothelial cell migration in a modified Boyden chamber in vitro (Fig.

Fig. 2. Secretion and accumulation of VEGF protein in CM after PDGF treatment. A, the amount of VEGF proteins analyzed by Western blotting was partially purified from 1 ml of CM by heparin binding. The anti-VEGF antibody recognized a major band with a molecular mass of 53 kDa, similar to that of VEGF165. Because VEGF165, which lacks heparin-binding domain, was not present in the purified samples, the minor band with smaller size may represent a proteolytic product of or a different glycosylated form of the VEGF165 (>). The larger minor may be VEGF189 (>). WT, wild type. B, for better quantitation, amount of VEGF proteins presented in CM was analyzed by VEGF ELISA using non-purified CM; bars, SD.
The CM from the PDGF-treated wild-type and the Y40/51 cells both stimulated PAE/KDR cell migration more than 2-fold over that of the CM from unstimulated cells. Neutralizing anti-VEGF monoclonal antibody suppressed the chemotactic activity of recombinant human VEGF and the CM to background level, but control IgG had no effect, indicating that VEGF was a major component in the CM responsible for stimulating PAE/KDR cell migration. In contrast, the CM from the F5, F40/51, or R634 cells had little stimulatory activity. Taken together, these data show that the levels of PDGF induction of VEGF mRNA and protein were correlated with the ability of mutant receptors to associate and activate PI3K, indicating that PDGF-induced VEGF expression is largely mediated by PI3K activity. We also...
showed that the amount of VEGF protein expressed by these cells was functional in its ability to stimulate endothelial cell migration.

**Dominant-Negative PI3K Inhibits PDGF-induced VEGF Expression.** To further confirm the involvement of PI3K in PDGF-driven VEGF expression, PAE/βPDGFR cells were transfected with plasmids expressing a well-characterized dominant-negative mutant of the p85 PI3K regulatory subunit, which lacks the binding site for the p110 catalytic subunit (p85ΔISH2-N; Refs. 38 and 39). Two independent clones were selected for having similarly high levels of expression of the exogenous p85ΔISH2-N protein and βPDGFR compared with clones expressing the empty vector (Fig. 9A). As shown in Fig. 9B, overexpression of the p85ΔISH2-N significantly blocked PDGF-induced VEGF mRNA, whereas expression of the empty vector had no effect. Consistent with the data obtained with the mutant receptors (Fig. 7A), dominant-negative PI3K constructs had little effect on relative levels of VEGF increases in response to exogenous PDGF because they also inhibited basal levels of VEGF mRNA that is caused by autocrine PDGF induction of VEGF expression, which apparently also requires PI3K activity. The inhibitory effect of the p85 mutant on VEGF induction appeared somewhat greater than that obtained by preventing activation of receptor-associated PI3K from F40/51 mutant receptor (Fig. 5A), perhaps suggesting its ability to interfere with upstream signaling molecules needed for activation of PI3K both by the receptor and by Ras, because Ras has been shown to be required for efficient activation of PI3K by PDGF (45).

**DISCUSSION**

Angiogenesis, the formation of new blood vessels, begins with activation of endothelial cells lining the inner wall of the parental vasculature in which VEGF plays an essential role. VEGF can be produced by tumor cells, macrophages, and within the vascular wall by vascular smooth muscle cells, and activates endothelial cells through a paracrine pathway. Here, we provide evidence suggesting that VEGF can also be produced within endothelial cells through activation of βPDGFR expressed on endothelial cells. We further established a mechanism for this by identifying a specific PDGF-mediated signaling pathway involving PI3K that modulates VEGF induction in endothelial cells.

**Fig. 5.** PDGF-driven VEGF expression was blocked by PI3K inhibitors. Serum-starved cells were preincubated with the indicated protein kinase inhibitors for 30 min and then stimulated with 50 ng/ml PDGF-BB or 100 ng/ml TPA for 2 h. Fifteen μg of total RNA was analyzed for VEGF mRNA signals by Northern blotting. A, two unrelated PI3K inhibitors, wortmannin and LY24009, were used at the indicated concentration. B and C: BIM, 5 μM bisindolylmaleimide GF 109203X, a PKC inhibitor; W, 100 nM wortmannin; LY, 20 μM LY24009.
The expression of \( b \)PDGFR on endothelial cells is a prerequisite for PDGF to elicit a direct effect on neovascularization. A large number of fresh samples of human astrocytomas of all grades examined by in situ hybridization and immunocytochemistry techniques showed that expression of \( b \)PDGFR was not detectable in the endothelial cells of normal human brain but was induced in the vasculature of low- and high-grade gliomas, particularly in the hyperplastic endothelium of glioblastoma multiforme (30, 31, 46). Up-regulation of \( b \)PDGFR has also been reported for the capillary endothelial cells in human carcinoid tumors, in wounds, and in inflammatory tissues (32, 47, 48). These observations suggest that PDGF may have a direct effect on endothelial cells undergoing angiogenesis. In this regard, PDGF may act as a mitogen and directly stimulate endothelial cell proliferation (49), or it may induce VEGF expression in endothelial cells, which in turn causes an autocrine stimulation through VEGF receptors. Here, we demonstrated that expression of \( b \)PDGFR in normal endothelial cells created an autocrine PDGF induction of VEGF expression, which was markedly augmented by exposure to exogenous PDGF. The VEGF proteins that were secreted were functional and capable of stimulating endothelial cell mitogenesis and migration. These results may be important to understanding the mechanisms of both autocrine and paracrine PDGF action in up-regulating VEGF expression in many pathological conditions where \( b \)PDGFR is induced in neovas-
cular endothelial cells. For example, this may explain the in vivo effect of PDGF on the development of a prominent blood vessel network in xenotransplanted human melanoma tumors (50). Studies of transgenic mouse models of tumorigenesis and human breast and cervical cancers have revealed that the angiogenic switch occurs in the early stages of tumor development, even preceding the appearance of solid tumors (51). In the case of glioma tumorigenesis, PDGF-induced VEGF expression may contribute not only to the expansion of an established tumor but also to the regulation of the angiogenic switch for initial tumor development, because induction of βPDGFR on endothelial cells can occur in early stages of glioma formation (30, 31, 46). These data emphasize the importance of epigenetic changes such as up-regulation of PDGF and its receptors in regulating angiogenic switch in the early stages of tumor development, as opposed to hypoxia, which may be a major stimulus for neoangiogenesis in an established tumor where the hypoxic condition becomes evident.

These findings extend the actions of VEGF from its usual paracrine mechanism whereby tumor cells secrete it to stimulate endothelial cell migration and proliferation. There is substantial evidence to indicate that endothelial cells themselves are capable of expressing VEGF as well. VEGF transcripts have been identified in several cultured endothelial cells such as rat brain capillary endothelial cells, bovine glomerular and retinal endothelial cells (52–54), and in diabetic neovascular membranes (55). Although many types of human endothelial cells express no VEGF under quiescent conditions, they can express VEGF in response to hypoxia (56, 57) and, as we showed here, in response to external sources of PDGF.

We have also established PI3K as a mediator essential in βPDGFR-mediated signaling leading to VEGF induction in endothelial cells by demonstrating that PDGF-induced VEGF expression was blocked by the PI3K inhibitors, wortmannin and LY24009, and by the dominant interfering p85 subunit of PI3K. This was further supported by the observations that whereas a mutant receptor that does not activate PI3K as well as three other receptor binding proteins expressed a minimal level of VEGF, restoring the PI3K binding sites to the receptor rescued nearly complete VEGF induction. We also showed that this induction was independent of PKC, in contrast to a previous report that NIH3T3 mouse fibroblasts cells require PKC for PDGF-dependent VEGF expression (19), possibly due to cell type specificity. It is also noteworthy that the induction of VEGF transcription by hypoxia in Ras-transformed NIH3T3 mouse fibroblasts appears to require PI3K as well (58).

Our data on PI3K-dependent VEGF induction in endothelial cells suggest a novel function for PI3K in angiogenesis. This complements and extends previous data that have implicated PI3K in tumor-promoting functions such as transformation, cell survival, anchorage-independent growth, and cell motility and invasion (59–63). It will be intriguing to investigate which of the downstream effectors of PI3K are involved in PDGF-dependent VEGF induction in endothelial cells. The known downstream effectors of PI3K include several PKC isozymes (PKCe, PKClα, and PKClδ), ribosomal S6K, the serine/threonine kinase Akt (also known as PKB-α and RAC-α), and the small G protein Rac, each of which mediates distinct biological responses (64). Recent reports also suggest that PI3K may interact with the Raf/mitogen-activated protein kinase pathway leading to gene regulation (65). In any case, the present studies suggest that under certain pathological conditions, VEGF can be activated through the activation of βPDGFR expressed on endothelial cells, thus modulating endothelial cell functions through an autocrine pathway that may enhance the paracrine effects of VEGF in stimulating angiogenesis. This induction is largely mediated by the PI3K signaling pathway, suggesting that PI3K plays a role in angiogenesis and is therefore a potential target for therapeutic inhibition of angiogenesis and tumorigenesis.

ACKNOWLEDGMENTS

We thank Drs. L. Claesson-Welsh and C. H. Heldin for the PAE and PAE/KDR cell lines, Drs. M. D. Waterfield and J. Downward for the plasmids of p85ΔSH2-N in pSG5, and members of the laboratory for critical reading of the manuscript.

REFERENCES


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